Remarks

Amendment of the Claims

In light of the Examiner's remarks and rejections, the Applicants request the Examiner's review of the amended Claims 53, 63, 64, 87, 89, 90, 91 and 93, previously presented Claim 92 and new Claims 94-98.

The Applicants have reviewed the Examiner's remarks and submit that the Claims presented herein are now in a condition for allowance, particularly in view of the Examiner's remark that the claims in the case are "enabling for a nucleic acid encoding 57 amino acid residue T7B peptide linked to the carboxy terminal of a protein of interest under physiological conditions and enhancing the protein's solubility and proper folding in *E. coli*".

Claim 53 has been amended to include limitation of the expression vectors to those that are optimized for use in prokaryotic cells and limitation of the peptide extensions to T7 gene 10B carboxyl terminal peptide extensions. The Applicants submit that this amendment properly addresses the Examiner's 35 USC 112 rejections (see further remarks below) and respectfully request that the rejections be withdrawn.

As will be noted by the Examiner, amended Claims 90 and 93 include the limitation of expression vectors to those that are optimized for use in bacterial cells.

Cancellation of claims 54-60 necessitated the amendment of the dependency of some of the other claims remaining under consideration, i.e., claims 63, 64 and 87.

Claims 88 and 89 and 91 are amended herein to more particularly point out and distinctly claim the subject matter of the invention.

The Applicants further request the Examiner's consideration of the new Claims, 94-98. One of skill in the art would predict that the peptide extensions of the present invention would have solubility and folding enhancement properties regardless of which prokaryotic cell hosted the expression from a vector optimized for use therein.

The Applicants submit that none of the amendments nor the new Claims contain new matter.

The following remarks are offered to persuade the Examiner that the application is now in condition for allowance.

Claim rejection – 35 USC 112

1. Rejection under 35 USC 112, first paragraph, written description:

Claims 53-60 have been rejected for failing to comply with the written description requirement.

In the current paper, the Applicants present an amendment of Claim 53 for the Examiner's consideration. In addition, the Applicants have cancelled Claims 54 – 60. The Applicants respectfully submit that as currently amended Claim 53 now complies with the written description requirement of 35 USC 112, first paragraph and is in condition for allowance.

The current amendment of Claim 53 contains no new matter.

2. Rejection under 35 USC 112, first paragraph, enablement:

Claims 53-60, 62-64 and 87-93 have been rejected because the specification fails to provide enablement for:

> "any peptide extension of 1 to 61 amino acid residues having a –2 to –22 net negative charge linked to any target protein and expressed in any host to enhance protein folding and solubility when expressed in any cell".

However, the Examiner also notes that the specification is:

> "enabling for a nucleic acid encoding 57 amino acid residue T7B peptide linked to the carboxy terminal of a protein of interest under physiological conditions and enhancing the protein's solubility and proper folding in *E. coli*".

In response the Applicants submit that the specification provides enablement for Claims 53 and 63, 64 and 87-93, as amended, as presented on pages 2-7 herein as well as for newly presented Claims 94-98, pages 7-8.

As noted above, the Examiner has pointed out that the specification is enabling for "a nucleic acid encoding 57 amino acid residue T7B peptide linked to the carboxy terminal of a protein of interest under physiological conditions and enhancing the protein's solubility and proper folding in *E. coli*". The Applicants respectfully request the Examiner's consideration that any prokaryotic cell expression vector or vector optimized for use in bacterial cells, in its proper prokaryotic/bacterial host cell would be expected by one of skill in the art to function similarly to the vectors in the examples which made use of *E. coli* cells and vectors. Thus the new claims, the Applicants submit, are fully enabled by the specification and are in condition for allowance.

Claim rejection – 35 USC 102

4. Claims 53-60 have been rejected under 35 USC 102(b) as being anticipated by Rechsteiner et al. (US Patent No. 5,366,871), the '871 patent.

In response, first, the Applicants request the Examiner's consideration that amended Claim 53 is not anticipated by Rechsteiner and is now in condition for allowance. Further, since claims 54-60 have been cancelled, the Applicants present the following remarks to persuade the Examiner that Rechsteiner does not anticipate the present invention and that the rejection should be withdrawn.

The amended Claim 53 clearly shows the novel and non-obvious difference between the present invention and the teachings of the '871 patent. It is abundantly clear that the T7 gene 10B carboxyl terminal 57 amino acids are materially different in structure and sequence from any of the peptide extensions of the '871 patent.

Further, as to the "functional characteristics" of the claimed products that differ from the teachings found in the '871 patent, there are clearly no teachings in the reference that would suggest that the '871 patent peptide extensions contribute to enhancing the solubility or folding of the "protein of interest" (ubiquitin). In fact, as noted in the '871 patent ubiquitin was chosen as the "protein of interest" (i.e. as the fusion partner) because:

> "Ubiquitin is **an extremely soluble protein** that can be expressed to very high levels within *E. coli* cells . . ." (emphasis added) (Col 2, lines 53-54)

Thus the '871 peptide extensions are substrates, and are not solubility/folding enhancers. In other words, the peptide extensions of the '871 patent cannot, in the words of the Examiner "be thought of as a solubilizing partner".

The Applicants submit that the amendment of Claim 53 to specify an expression vector optimized for use in prokaryotic cells serves to eliminate from further discussion whether or not the peptide extensions of the present invention would serve similar solubility and folding enhancement in other cells, such as eukaryotic cells. Despite this, the Applicants wish to address the Examiner's remark about expression of the '871 peptide extension in eukaryotes. The Examiner states: "Expression is observed in prokaryotes (e.g. col. 9, Example 2) **or it can also be in eukaryotes** (e.g. col. 13, Example 10)." The Applicants respectfully wish to point out that the Examiner has misinterpreted this element of the teachings of Rechsteiner as follows.

The '871 patent teaches methods for assaying various peptide-modifying cellular enzymes such as kinases, farnesyl-transferases, etc. The substrates for these assays are comprised of the protein of interest (i.e., ubiquitin) fused to a peptide extension "wherein the peptide contains a sequence known to be modified by an agent in the presence of the enzyme being assayed for". The vectors of the '871 patent were designed to incorporate specific peptides that are known to be modified by an enzyme such as a kinase or farnesyl transferase onto the carboxyl terminus of ubiquitin. Rather than produce the "fusion proteins" synthetically the inventors prepared them by modifying the genome of the **bacteriophage** M13 and having the ubiquitin fusion

protein expressed following heat induction of bacteriophage-lysogenized *E. coli*. Thus, the inventors induce expression of the modified ubiquitin in E. coli, isolate the ubiquitin fusion protein and then use it as a substrate to assess the activity of specific peptide-modifying enzymes found in various cellular extracts.

Because the M13 vector is a **bacteriophage** it **cannot** be grown in eukaryotes. Thus, expression of the '871 fusion protein cannot take place in eukaryotes. In Example 10 of the '871 patent a ubiquitin fusion protein, expressed in *E.coli* from a recombinant **bacteriophage M13,** is isolated and then used to assay for peptide-modifying kinases found in extracts of HeLa cells (human) mouse liver (murine) and Xenopus eggs (amphibian).

Claims 53-60 are rejected under 35 USC 102(b) as being anticipated by Barnhart et al. (PNAS 2000; 97:709-14) – "Barnhart".

The Examiner states:

> "Barnhart teaches nucleic acid constructs in an expression vector that encode a fusion protein where a 13-amino acid peptide extension is added to the C-terminal end of a protein of interest. (. . .) The peptide extension sequences are shown to enhance folding under physiological conditions in Gram-negative bacteria." (emphasis added)

The Examiner further notes that:

> "the burden is on the applicant to show a novel or unobvious difference between the claimed products and the products of the prior art (. . . . the products of the prior art do not possess the same material structural and functional characteristics of the claimed product)."

In response, first, the Applicants request the Examiner's consideration that in view of the present amendment of Claim 53, cancellation of claims 54-60, and the following remarks, the present invention is not anticipated by Barnhart.

The Applicants respectfully wish to draw the Examiner's attention to the novel and unobvious material structural and functional differences between the claimed products and the products of the prior art.

First, it is clear from comparing the sequence of the peptide extension of Figure 1B of Barnhart with the sequences of the claimed T7 gene 10B peptide extension that there is no similarity with respect to sequence. Furthermore, the T7 gene 10B peptide has a net negative charge of –6 under physiological conditions. Using a similar method for determining net charge under physiological conditions the DNKQ – DVTITVNGKVVAK (the 4 amino acid "hairpin loop" and the 13 amino acid residues from FimG that form the "peptide extension") peptide extension has a net charge of +2 for the 13 amino acid extension and, additionally considering the hairpin loop, the entire peptide extension has a net charge of +4 as follows:

```
 D   N   K   Q     D   V   T   I   T   V   N   G   K   V   V   A   K
-1  +1  +1  +1    -1   0   0   0   0   0  +1   0  +1   0   0   0  +1  = +4
```

Thus, not only is the peptide of Barnhart unrelated in sequence to the peptides of the present invention, the net charge is completely different from the charges of the peptide extensions of the present invention.

In addition, the Applicants draw the Examiner's attention to the structural conformation differences between the peptides of the present invention (unstructured,

non-ordered, random) and the peptide of Barnhart (a combination of a hairpin loop and a β-strand of canonical Ig folds).

The T7 gene 10B peptides are unrelated to the Barnhart peptide, which peptide consists of a hairpin loop region (Asp-Asn-Lys-Gln; i.e., DNKQ) and the first 13 amino acids of FimG. (Col. 1, page 7710, last paragraph) The 13 amino acids of the N terminus of FimG (Asp-Val-Thr-Ile-Thr-Val-Asn-Gly-Lys-Val-Val-Ala-Lys; i.e., DVTITVNGKVVAK) form the "seventh C-terminal β-strand (strand G) present in canonical Ig folds". (see Col. 1, page 7709, second paragraph) Further, per the authors: "We investigated whether we could alleviate the need for a chaperone by providing the missing strand in cis, fusing the missing seventh β-strand onto the 3' end of *fimH*. The addition of the N-terminal extension of FimG onto the C terminus of FimH (. . . .) resulted in the production of a protein that was now stable in the periplasm . . . .". (Col. 2, page 7709, 1st full paragraph) Taken together this suggests that the structural fold adopted by the FimG peptide extension (seventh β-strand of Ig folds) acts to chaperone FimH into a properly folded molecule. This suggests that the FimG extension must form a non-random, ordered conformation in order to act in this manner. **By contrast,** the peptide extensions of the present invention are non-ordered, unstructured and random in conformation as discussed throughout the specification and claims (e.g., see page 8, lines 13-15; page 9, lines 13-16; and originally filed Claims 9, 14, 35, 49, 53, 65).

Thus, the Applicants submit that in light of the above remarks and the amendments of the claims in this case, the Barnhart reference is moot and the claims in the present case are now in condition for allowance.

Summary

Claims remaining under consideration include currently amended Claims 53, 63, 64, 87, 89, 90, 91 and 93, previously presented Claim 92 and new Claims 94-98. Neither the amendments of the Claims nor the new Claims contain new matter.

In light of the above Amendments and Remarks, applicants respectfully submit that the instant application is now in condition for allowance and solicit a timely notice of allowance.

Respectfully submitted,

Christine L. Brakel
Agent for Applicants
Registration No. 45,772

Date: February 6, 2006

Christine L. Brakel
Patent Agent
Brookhaven National Laboratory
Bldg. 475D
P.O. Box 5000
Upton, New York 11973-5000
(631) 344-7134